HETEROGENEITY OF FACTOR IX IN THERAPEUTIC CONCENTRATES. G. Monarch and D. Arosen, Hôpital Beaumont, Clichy, France; Bureau de Biologies, Bethesda, Maryland, U.S.A.

In vivo and in vitro experience shows that factor IX concentrates are partially activated. A rabbit antiserum to human factor IX was used to investigate the factor IX antigenic content and electrophoretic mobility of commercial products as well as experimental “activated” preparations. Electrophoresis of all concentrates showed a 1.5-3 fold increased antigenic content/unit factor IX clotting activity when compared to plasma. Two-dimensional crossed immune-electrophoresis of standard factor IX preparation produced a single sharp peak whether electrophoresis was carried out in Tris-HCl containing buffer. “Activated” preparations produced a dome shaped precipitin arc. The addition of plasma to factor IX concentrates yielded a marked shoulder only when the electrophoresis was done in EDTA. This effect could not be reproduced by the addition of antithrombin III (AT-III). The addition of plasma to the activated IX (IXa) revealed an even more pronounced heterogeneity whether in Ca2+ or EDTA. The addition of AT-III produced a second precipitin peak when activated IX was electrophoresed in the presence of Ca2+.

These results indicate that at least three forms of factor IX exist in factor IX preparations. The absence of detectable AT-III reacting material in the regular IX preparations is a prior evidence of the absence of major amounts of IXa, whereas the presence of AT-III reacting material in the “activated” complex is evidence of biologically active material.

CIRCULATING FACTOR IX ANTIGEN-INHIBITOR COMPLEXES IN HEMOPHILIA B FOLLOWING INFUSION OF A FACTOR IX CONCENTRATE. S.H. Goodnight, C.M. Britell, E.R. Waeger and R. Buxtorf, University of Oregon Health Sciences Center, Portland, Oregon and University of California at San Diego, San Diego, California, U.S.A.

A persistent low titer IgG factor IX inhibitor (0.8-1.3 U/ml) has been previously described in an 11 year old boy with severe hemophilia B. Since the disappearance of factor IX antigen (IXαg) following Bonytin infusion was markedly delayed (75 60 hr) compared to factor IX activity (25-60 hr), a search was made for circulating factor IX antigen-inhibitor complexes was undertaken. The inhibitor binds firmly to staphylococcal protein A-Sepharose 4B (SPA-Sepharose) but may be eluted in the IgG 1-2 and 4 fraction with 3M NaCl. SPA-Sepharose chromatography of post-infusion plasma (IXαg 2.7 U) from the patient with the inhibitor showed IXαg (0.5 U) in the IgG 1-2, and 4 fraction, whereas IXαg was not detected in that chromatographed samples of post infusion plasma from non-inhibitor patients with hemophilia B. Using a highly specific antibody to factor IX, two dimensional immuneelectrophoresis of purified factor IX or normal plasma showed a single symmetrical fast moving peak. When an equal volume of inhibitor plasma was added to either factor IX or normal plasma a second, slower moving component was also seen. Two dimensional immuneelectrophoresis of plasma obtained from the patient 43 minutes after infusion showed both the slow and fast components. The presence of IXαg in the IgG 1-2, and 4 peak after SPA-Sepharose chromatography and the absence of IXαg on the dimensional immuneelectrophoresis indicates the presence of circulating factor IX antigen-inhibitor complexes in the plasma of this patient following the infusion of a factor IX concentrate.

FACTOR Xa LIKE ENZYME INSnake "HABU" VENOM. I. Matsumura, T. Kinoshita, M. Kazama, A. Igata and T. Abe, Kagoshima University School of Medicine, Kagoshima and Kitakyushu School of Medicine, Itabashi, Tokyo, Japan.

The snake "HABU" venom was divided into subfractions and their influence on both platelet and coagulation was studied. Four subfractions were obtained by gel exclusion chromatography on Sephadex G75 of which molecular weights ranged from 10,000 to 5,000. The fraction of the largest molecular weight was further divided into four subfractions: 1-3, 1-2, 1-3 and 1-4 by DEAE cellulose chromatography. The subfraction 1-3 showed no activity to induce platelet aggregation but suppressed ADP, collagen and thrombin-induced aggregation. The subfraction 1-2 and 1-3 equally induced the aggregation of plasma platelet and gel filtrated platelet suspension. The subfraction 1-4 had the same effect on the platelet aggregation except for the specific suppressive effect on the thrombin aggregation. The subfraction 1-3 was found to have Xa-like activity which was revealed using chromogenic substrate (S-2222). This activity was not inhibited by purified human antithrombin III, O-macro-globulin or O-antitrypsin. The relationship between activities of these fractions on platelet and coagulation suggested that they are different from any known coagulation factor.

HABU -- Trimeresurus flaviviridis Hallowell